



Editorial: Understanding C₄ Evolution and Function

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Editorial on the Research Topic

Understanding C₄ Evolution and Function

C₄ photosynthesis is a remarkable example of convergent evolution, having independently evolved at least 62 times over the last 60 million years (Sage et al., 2011). In C₄ species, Rubisco operates close to its maximal carboxylation rate through suppression of the oxygenation reaction. This activity is accomplished via the establishment of a molecular CO₂ pump that delivers carbon in the form of C₄ acid intermediates to a spatially sequestered Rubisco. This carbon pump can be set up using a diverse array of complex biochemical and morphological modifications relative to the ancestral C₃ photosynthetic state.

The large number of independent origins of a C₄ syndrome suggests that evolution from ancestral C₃ photosynthesis to a derived C₄ type is flexible at the molecular level and relatively easy in genetic terms (Gowik et al., 2004; Williams et al., 2013; Heckmann, 2016). With a large pool of biodiversity to exploit, such as in Southwest Asia, reviewed here by Rudov et al., natural variation in diverse phylogenetic lineages can be used to better understand the molecular changes enabling evolution of a functional C₄ syndrome. The papers presented in this Research Topic make use of this biodiversity to expand our knowledge of C₄ evolution and function.

Despite C₄ photosynthesis being highly convergent, little work has been done to understand which C₄ traits have arisen through convergence and could be considered essential for a C₄ syndrome. Here, Khoshravesh et al. use gas exchange, leaf ultrastructure and biochemistry and carbon isotope ratios to characterize the carbon assimilation pathways used by species in the eudicot family Nyctaginaceae, and in the case of the C₄ members, to determine the subtype of C₄ photosynthesis. Combining these data with those from other C₄ clades, they compiled a hierarchical list of convergent and divergent traits.

Gene duplication has been proposed as one of the early steps in the recruitment of genes during evolution of a C₄ pathway (Monson, 2003). Tronconi et al. describe a complex evolutionary history responsible for present-day C₄-associated NAD-malic enzyme (NAD-ME) in the Brassicales that involves ancestral gene duplication followed by degeneration, complementation subfunctionalization, and neofunctionalization. Gene duplication and co-option also appear to be responsible for the evolution of the C₄-associated PEP transporter, PPT1. Lyu et al. identify differences in coding and non-coding regions between C₃ and C₄ orthologs of PPT1 associated with increased expression of the transporter in C₄ mesophyll cells (MC). They find that gene duplication and neo-functionalization led to recruitment of a PPT1 paralog found in roots to a role in C₄ function.

Most C₄ species operate a carbon pump with the help of Kranz anatomy, wherein MC surround highly specialized bundle sheath cells (BSC) that are concentrically arranged around the vasculature

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(Sage et al., 2014). In a small number of species, special organellar arrangements within a single cell are used to achieve high CO₂ concentrations around Rubisco (Sharpe and Offermann, 2014). In work on *Suaeda aralocaspica*, a single-cell C₄ species, Cao et al. identify paralogs encoding the C₄-associated phosphoenolpyruvate carboxylase (PEPC), which catalyzes the first step in the C₄ pathway, a housekeeping isoform, and a bacterial-type PEPC.

Given the apparent flexibility of gene recruitment during evolution of C₄ syndromes, identification of regulatory components controlling the spatial expression of C₄-associated enzymes is important for understanding C₄ function. Here, Afamefule and Raines use C₃ and C₄ grasses to screen upstream regions of genes encoding four enzymes in the Calvin-Benson-Bassham (CBB) cycle for conserved nucleotide sequences that might enable cell-preferential expression. They identify *cis*-regulatory elements putatively involved in BSC-enriched expression of genes encoding CBB enzymes as well as candidate transcription factors potentially binding to those sites. In addition, Górska et al. identify three *trans*-acting factors that bind the upstream region of the C₄-associated PEPC homolog of maize. Characterization of these factors highlights the complexity of cell-preferential expression in a C₄ leaf and the role of repression in establishing some C₄-type expression patterns.

Of course, evolution is ongoing. As suggested by the results of Moody et al. in a study on PEPCs from older and younger C₄ lineages, optimization of the enzyme continues after a C₄ syndrome is realized. Similar comparative studies of other enzymes in the C₄ acid cycle may also contribute to our understanding of how a C₄ syndrome evolves at the molecular level.

A better understanding of the molecular events underpinning evolution of a C₄ syndrome could enable a C₃ plant to be engineered for C₄ features. This is highly desirable because C₄ crops have higher yields and increased nitrogen and water use efficiency relative to C₃ crops. Replicating the C₄ process in C₃ crops such as rice would therefore help feed a growing world population. Support for introducing a C₄ pathway into rice is provided by Lin et al. Genes encoding four of the

major enzymes in the maize NADP-ME-type C₄ pathway, PEPC, NADP-malate dehydrogenase (NADP-MDH), NADP-ME and pyruvate phosphate dikinase (PPDK), were inserted into the rice genome. Subsequent measurements with ¹³C demonstrate that production of ¹³C-labeled malate was high in the transformants, suggesting that a partial C₄ pathway is functioning in these plants.

Studies on C₄ physiology and metabolism are also important to improve breeding programs of C₄ crops. In particular, light harvesting and nutrient availability and uptake are key determinants for crop productivity. Collison et al. explore relationships between leaf age and light availability with the phenomenon of shade maladaptation exhibited by the NADP-ME-type C₄ crops maize, sorghum and sugarcane. Leaf age had little influence on the quantum yield of CO₂ assimilation. Instead, optimization of the leaf light environment mitigates the negative effects on productivity associated with this maladaptive response. These results can inform breeding strategies related to canopy structure and agricultural practices such as planting densities to increase crop yield.

Jobe et al. highlight the need to consider the nutritional value of C₄ crops in addition to yield. They review nutrient assimilation pathways in C₄ plants and how they differ from C₃ plants as well as discuss gaps in our knowledge of how nutrient uptake and levels are controlled in C₄ plants. They also consider the effects of increasing atmospheric CO₂ on C₃ and C₄ crop micronutrient assimilation and content in light of micronutrient-related malnutrition (i.e., hidden hunger). Such considerations are important for producing future C₄ crops that will effectively address global food needs.

In summary, this collection of articles expands our understanding of C₄ evolution and function. This new knowledge will inform future work in evolutionary biology, C₄ metabolism, and crop improvement strategies.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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